Methods for stabilizing protein

Abstract

In basic research and drug discovery there is a need to provide protein reagents with high activity. In particular proteins that are used in assays must have a good shelf life and a consistent activity.
As a system of measuring actin polymerization, pyrene labeled actin was developed in 1983 by Kouyama and Mihashi. However, prior to this disclosure, pyrene actin has been impossible to store for longer than one month because the activity would be lost due to protein denaturation and frozen pyrene actin immediately lost activity. This disclosure relates a method for stabilizing pyrene labeled actin for extended storage times and an optimal storage buffer thus creating a reproducible source of pyrene actin for various assays. Using the processes described herein, pyrene actin has been stored for greater than 3 years at 4°C which makes it a viable product for retail.

Claims

Claim 1. A process of stabilizing pyrene actin which requires concentrating the labeled protein to greater than 10mg/ml, preferably greater than 20mg/ml and most preferably greater than 30mg/ml prior to freezing and lyophilization.

Claim 2. A process of stabilizing pyrene-actin that incorporates the essence of Claim 1 and additionally requires rapid freezing prior to lyophilization, preferably in a dry ice ethanol bath, and most preferably in liquid nitrogen.

Claim 3. A process of stabilizing pyrene actin's activity to be more like the freshly prepared form, by including high amounts of a reducing agent prior to lyophilization.

Claim 4. A formula for pyrene actin lyophilization that allows storage of pyrene actin for greater than 3 years at 4°C, which is greater than 10mg/ml pyrene actin, preferably greater than 20mg/ml pyrene actin and most preferably greater than 30mg/ml pyrene actin, plus 5mM Tris-HCl pH8.0, 0.2mM ATP, 0.2mM CaCl2, 5% w/v sucrose, 1% w/v dextran and 10mM DTT.

Claim 5. A method of reconstituting lyophilized pyrene actin so that it recreates the character of freshly prepared pyrene actin, which includes resuspending pyrene actin to 0.4mg/ml in A-buffer (5 mM Tris pH8, 0.2mM CaCl2, 0.2mM ATP, also called G-buffer) and incubating for 1h on ice to depolymerize oligomers of actin that form during the preparation process, and preferably an additional 100,000 x g centrifugation for 2h to remove any remaining oligomers, prior to performing polymerization studies.